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Dietary Habits and Folate Status in Women of Childbearing Age in Croatia

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ABSTRACT

Folic acid and folates have an important role in prevention of neural tube defect that appears in the first weeks of pregnancy, when women are still not aware of their pregnancy, especially when pregnancy is not planned. Since ensuring sufficient quantities of folates and folic acid in this period is essential, dietary habits of childbearing age women are very important. In line with that the intake of folates and folic acid in nutrition of women age group 20–30 years is examined, as well as the frequency of consumption of foodstuffs rich in vitamins and folic acid supplements. Values of folates in serum are presented, dependent on their nutritional habits. Obtained results indicate that in spite of inadequate intake of folates and folic acid from foodstuffs, clinical deficit is not recorded, which is the result of frequent consumption of dietary supplements. In accordance with these dietary habits, differences in the folates status of examinees were observed.

Key words: *nutrition, folic acid, dietary habits, women*

Introduction

Based on animal studies, epidemiological studies and intervention trials, maternal folic acid is known to be protective for neural tube defects (NTD)[1,2–4]. NTDs are birth defects that include spina bifida, anencephaly, encephalocele or iniencephaly, which are devastating, sometimes even fatal birth defects that occur between the 21st and the 27th days after conception, a time many women do not realize yet that they are pregnant.

In primary prevention, since about half of pregnancies are not planned, birth defect prevention include a recommended daily dose of 400 μg synthetic folic acid for women of childbearing age and 600 μg for pregnant women[5,6].

According to the hospital morbidity indicators in Croatia, during 2003 there were 45 hospitalized cases of spina bifida (MK X.rev.Q05), of which number 16 were under one year old; 858 hospital days were utilized for the treatment of these patients, while for children under one year only 357 hospital days were spent. Thus, the treatment of spina bifida represents significant health expenditure. Although the prevalence of spina bifida in the Croatian population has not significantly increased, it is not to be neglected, particularly if it is possible to reduce the incidence by public health interventions, i.e. by adequate nutrition.

In the United States one per 1,000 pregnancies result in neural tube defects such as spina bifida and anencephaly in newborns, as a consequence of inadequate nutritional intake of folate in the population of women of childbearing-age. Estimated mortality rate of infants with spina bifida in the United States is 10%, in the Netherlands 35%, while in poor rural areas of northern China it comes up to 100%, which represents one of very important public health problems[7].

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Numerous researches found out that taking folic acid could prevent 50–70% of the above-mentioned cases[8–10].

The findings led the UK Department of Health to conclude and recommend that women of childbearing age should consume an extra 400 μg folic acid or folate per day to prevent NTD[11]. Other national committees including the United States' Centre for Disease Control and Prevention and the Australia's National Health and Medical Research Council[7] have made similar recommendations.

Women at high risk for having a child with a NTD (e.g. a previously affected child) should consume 10 times greater amount of folic acid (4 mg per day) at least three months prior to conception and through the first trimester of pregnancy.

Folate also plays an important role in metabolism of amino acids. Thus, folate deficiency can result in decreased synthesis of methionine and build up of homocysteine, which high levels are a risk factor for heart disease and stroke[12–16]. Folate is needed to make DNA and RNA, lack of it leads to megaloblastic anemia[17,18]. Folate is necessary in methylation of DNA, which also prevents changes in DNA that may lead to cancer[19]. Folate is especially important during periods of rapid cell division and growth such as during pregnancy[1] and infancy. George at al[20] found that folate deficiency is a risk factor for spontaneous abortion. During pregnancy, low concentrations of dietary and circulating folate are associated with increased risks of pre-term delivery, infant low birth weight, and fatal growth retardation[21].

Folic acid is the synthesized stable oxidized form of an essential water-soluble B-complex vitamin that occurs naturally as various folates, usually in reduced, conjugated forms. Folic acid is used in supplement tablets and food fortification (enriched breads, cereals, flours, corn meals, pastas, rice, and other grain products), while folates are found naturally in foods[22]. Rich food sources of folate are: leafy greens such as spinach, lettuce and turnip greens, dry beans and peas, tomatoes and oranges[5].

The folate intake can be increased by a diet, by folic acid supplements or by fortification of food with folic acid[6].

There is no folate consumption from dietary sources or folic acid supplements data available and there is also a lack of recent data on folate status of Croatian women. Therefore, the aim of this study was to assess the dietary intake of folates and folic acid and to determinate the serum folate values of childbearing age women in Zagreb, Croatia. Such baseline data are important for possible local public health actions and comparison with EU's and other countries' trends.

Materials and Methods

Sample selection

The study group consisted of 100 women of childbearing-age (range 20–30 years), mean age 24±3.7 (X± SD). The subjects were residents of Zagreb and its surroundings and were included in this study according to their admission to the outpatient Student's Polyclinic in Zagreb. Pregnant women and women who were on special diet, as well as those ones with previous medical history or long lasting therapy were excluded. Ethical Committees of Zagreb University School of Medicine approved the study protocol. Informed written consent was obtained from all participants in the study.

Questionnaires

Assessment of dietary folate and folic acid intake was carried out on a sample of 100 women who reported 24-hour dietary records during two days (first time admission to outpatient clinic and second time during check up). The 24-hour recall included photographs of meals for estimation of portion sizes. The portion sizes were presented in three sizes: I-small, II-medium, and III-large[23]. The folate/folic acid values of diet were estimated using a computerized food composition table (Food Data Systems software: Croatian version of NutriGenie total nutrition for Professional Windows 2001).

This questionnaire was also designed to identify nutrient intake from fortified, ready-to-eat breakfast cereals. Enriched cereals that appeared later on the market, but are not included in the food composition database, were also recorded. In all subjects included, mean folate and folic acid intakes were assessed through food intake, excluding supplements of folic acid. Folate values reported here are based on the average for two 24-hour dietary records for each subject. Besides using the 24- hour recall method during the two consecutive days, the Food frequency questionnaire (FFQ) was applied as well.

FFQ was elaborated in accordance with the tables of contents of foodstuffs and drinks[24]. The frequency consumption of folate rich foods was determined on the basis of reporting: every day, 4–6 times a week, 2–3 times a week, once a week, rarely, never. This questionnaire also identified nutrient intake from folic acid supplements (vitamin drinks) and from fortified, ready-to-eat breakfast cereals. The food was grouped in the following groups, as shown in the Figure 1.

Participants also answered questions about the intake of multivitamin supplements containing folic acid or single folic acid supplements on the basis of frequency: every day, sometimes, never. Amount of folic acid in vitamin supplements ranged from 100 to 800 μg with the median 250 μg.

According to the results obtained through dietary assessment, participants were divided into 3 groups:

– Group A – subjects who consumed folic acid supplements as vitamin drinks and tablets;

– Group B – subjects who consumed folic acid supplements, either as vitamin drinks or tablets;

– Group C – subjects who did not use folic acid supplements.

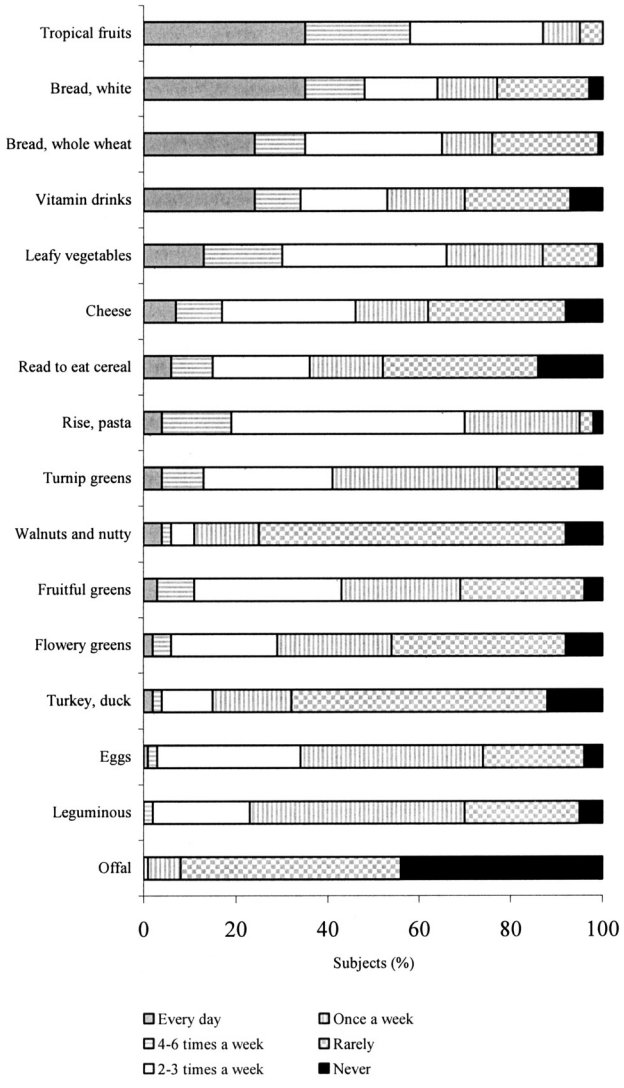

Fig. 1. Frequency of consumption of foodstuffs rich in folate and folic acid.

Serum folate analyses

The folate concentrations in blood were determined from blood samples of random sample of 100 healthy women, aged 20–30 years. The folate concentrations were identified by use of Abbott IMx System commercial kit (Folate, Abbott Laboratories, Abbot Park, IL, USA), according to the manufacturer's recommendations in the Institute of Laboratory Diagnostics, Zagreb University Hospital »Rebro«.

Statistical analysis

The results of the survey were presented for quantitative variables with mean values, standard deviation and 95% confidence interval, while the results of qualitative variables were presented in absolute (relative) frequencies (n=100) and were showed in tables. Testing for differences of serum folate according to the use of dietary folic acid supplements is performed using ANOVA and Tukey's post hoc test, and the data were analysed by the SPSS statistical package for Windows (version 9.0; SPSS Inc, Chicago, IL, USA). P values <0.050 were considered statistically significant.

Results

Our results based on the data obtained through 24-h recall show that the mean intake of naturally occurring food folate and folic acid from fortified cereals in 100 women of childbearing-age was 156.6±72.2 μg/day (Table 1).

TABLE 1
FOLATE INTAKE ASSESSED BY 24 HOUR RECALL AND SERUM FOLATE IN BLOOD

	X±SD	95% CI
Folate intake (μg)	156.6±72.2	142.3–171.6
Serum folate* (nmol/L)	23.2±9.2	21.2–25.3

*referent values: 7–28 nmol/L, 95%CI – 95% confidence interval

The results on frequency of consumption of foodstuffs rich in folate and folic acid are shown in the Figure 1: majority of the subjects (35%) daily consume white bread and citruses, and take folic acid supplement drinks (24%). Half of the subjects eat rice and pasta 2–3 times a week, while at the same time more than one third of them consume leafy and fruity vegetables and wholegrain bread. Eggs, root vegetables and leguminous vegetables are the foodstuffs which most of the subjects consume once a week. Nut products, duck and turkey meat, cereals enriched with folic acid, cheese, and flowery greens are rarely consumed. The subjects stated that they only rarely consume offal (48%), while 44% of the subjects have never consumed it. Our results on frequency of consumption of foodstuffs rich in folate and folic acid are in line with the results of the First Croatian Health Survey[25].

Significant increase in folate/folic acid intake is achieved by consumption of vitamin drinks that contain folic acid. Value of folate/folic acid intake in the whole day meal has increased from 173.1±14.3 μg to 379.3±24.8 μg in the subjects who consumed vitamin drinks on a daily basis. It is observed that one fourth of the subjects consume these drinks daily, almost half of them weekly, while only 7% of the subjects never use them.

Apart from vitamin drinks, intake of multivitamin supplements that contain folic acid, or only folic acid intake in form of tablets, has been recorded. According to our questionnaire, 21% of women did not take any folic acid supplement in the form of tablets, 53% took regularly, and 26% took vitamin supplements periodically.

Serum folate analyses

The folate status in all subjects was assessed by measuring of folate in serum. The folate in serum mean value

TABLE 2
SERUM FOLATE (NMOL/L) LEVELS WITH RESPECT TO VITAMIN SUPPLEMENTS CONSUMPTION

Group	X±SD	95% CI
A (n=20)	25.1±9.0	24–29
B (n=69)	21.7±5.7	18–23
C (n=11)	17.4±9.3	13–22
Total (n=100)	23.2±9.2	21–25

95% CI – 95% confidence interval, group A – subjects who consumed folic acid supplements as vitamin drinks and tablets; group B – subjects who consumed folic acid supplements, either as vitamin drinks or tablets, group C – subjects who did not use folic acid supplements.
$F=11.69_{(97;2)}$ $p<0.001$, ANOVA, Tuky HSD (significant difference test): difference among groups A and C ($p<0.001$), B and C ($p=0.040$), A and B ($p=0.485$).

is within the normal value range: 7–28 nmol/L, i.e. no clinical deficit is identified.

However, differences in concentrations of folic acid in serum of the subjects who consumed folic acid supplements are identified (Table 2). Thus, differences were found ($p<0.001$) between the Group A (subjects who consumed folic acid supplements in drinks and tablets) and the Group C (subjects who do not take them). There are also differences between subjects of the Group B (subjects who took folic acid supplements in only one form of drinks or tablets) and the Group C (subject who took folate only through foodstuffs, and did not consume folic acid supplements, $p=0.040$).

Discussion

This survey proved that the values of folate and folic acid intake in a whole day meal of the participants were less than 180 μg, which does not meet the criteria for anaemia prevention and particularly not recommendations for prevention of neural tube defects in newborn, respectively, in the periconception period.

Our results are in compliance with results of other authors and they show that nutrition of childbearing-age women, but also of the population in general, are inadequate from the point of view of folate and folic acid intake in the whole day meal. Thus Rimm at al[26] state that 88–90% of general population consume folate from foodstuffs in the amount less than 400 μg a day, while results of other researchers indicate that in childbearing-age women population this percentage is 68–87%[27]. Tarasuk and collaborators[28] also showed that folate intake from foodstuffs is not sufficient, amounting at 174±96.5 μg. Some greater folate and folic acid intake was recorded in the childbearing-age women in Germany[29], although it is still insufficient, and depends on kind and number of consumed foodstuffs (intake values range from 12 to 690 μg[30]). The lowest folate intake were recorded in the subjects who apart from poor consumption of foodstuffs rich in folate usually have reduced number of meals and an insufficient energy intake during day, although they are not on a diet.

Within the population of childbearing age women there are also differences concerning the folate and folic acid intake values in the whole day meal. In the population of women between 14 and 18 years, 85% of them consume less than 400 μg[31]. It has been observed that women in the age of 18 to 39 years have lower intake compared to the women of 40 to 46 years[22].

Apart from frequent consumption of foodstuffs rich in folate, through which the daily intake of 400 μg is very difficult to reach, substantial intake increase is possible to gain through frequent consumption of foodstuffs enriched by folic acid or folic acid supplements.

Therefore, it is recommended to all childbearing-age women to consume folic acid supplements[32], particularly taking into account bioavailability of folic acid, which is more than twice the bioavailability of folate available in foodstuffs[33]. Increase in folic acid intake of 0.1 mg a day increases concentration of serum value for 0.94 ng/mL of childbearing-age women[2].

Similar to our survey's results, Green and collaborators[34] showed that consumption of supplements lead to the increase of folate and folic acid, which was reflected in serum folate levels. Measuring concentration of folic acid in serum of the subjects who did not take folic acid supplements a median value amounted to 9.1 nmol/L, while the subjects who took folic acid supplement had a folic acid median value of 20.4 nmol/L.

Therefore, folic acid supplements consumption in form of drinks and tablets was favourably reflected on the subjects' folate status and there was not a case of clinical deficit in our survey. Similar surveys also show the absence of clinical deficit of folate in childbearing-age women, whereas upper ranges of concentration values amount to 45.3 nmol/L[35].

Results of the large research, the third National Health and Nutrition Examination Survey (NHANES III), which was carried out in the period from 1988 to 1994 and involved around 34,000 subjects, showed that approximately 29.2% of the United States' population, and 33.2% of childbearing-age women used at least 1 product in the previous month that contain folic acid. Moreover, during quantification of folic acid use, 62.1% of all folic acid consumers (of which percent, 67% are women and 55% men) have stated that they consume at least 12,000 μg/per month of folic acid (equivalent to 400 μg/day). Among them 71% are childbearing-age women who consume that recommended amount[36]. These results indicate significant use of folic acid supplements, which is sufficient to prevent clinical deficit that appears after more than four months of inadequate folate intake[33], which is in accordance with our results.

Since synthetic folic acid has twice the bio-availability of naturally occurring folate and most pregnancies are unintended, the USA's Institute of Medicine recommends 400 μg/day of synthetic folic acid in addition to a varied diet[30]. Other countries recommend 400 μg of folate daily

obtained through diet, and/or supplements (Finland, Sweden), while some countries introduced folic acid fortification (USA, the Netherlands)[6].

There are several levels of public health response through development of nutritional habits of consuming foodstuffs rich in folate, taking foodstuffs enriched by folic acid and supporting habits of taking folic acid supplements.

However, only public health recommendations to the childbearing-age women, as other countries' experiences show, are very often inefficient (USA, UK). Therefore, a public health action of obligatory enrichment by folic acid should be taken into consideration, since by this means average daily folate and folic acid intake would increase up to 100 μg[5,12]. In similar manner the Food and Drug Administration (FDA) introduced in 1996 legal regulation of obligatory enrichment of foodstuffs by folic acid, along with iron, thiamine, riboflavin and niacin that are already added in the cereal products (flour, rice, bread, bakery products and pastries, oatmeal, corn flakes, pastas), which came into force in the United States on 1 January 1998[37], and that example was followed by Canada[38].

The above-mentioned public health measures should have a protective impact on the persons with a higher risk to develop cardiovascular diseases (with the objective to decrease the level of homocysteine), with a higher risk to develop malignancy (preventive effect of folate) and persons with a higher risk for development of Alzheimer disease and deterioration of cognitive abilities (preventive effect of folate).

In order to get better insight into dietary habits and nutritional status of childbearing-age women in Zagreb as well as in whole Croatia, the number of subjects should be enlarged, and the survey itself should be extended to the whole of Croatia, taking into account different geographical regions that have impact on nutritional habits of the population. In compliance with the aforementioned, the survey is planned to continue with the objective to ascertain the need of introducing fortification of foodstuffs with folic acid in our country.

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PREHRAMBENE NAVIKE I FOLATNI STATUS ŽENA GENERATIVNE DOBI U HRVATSKOJ

S A Ž E T A K

Folna kiselina i folati imaju značajnu ulogu u prevenciji defekta neuralne cijevi koji se javlja u prvim tjednim trudnoće kada često žene ni ne znaju da su trudne, naročito ako je riječ o neplaniranoj trudnoći. Obzirom da je upravo u tom razdoblju nužno osigurati dovoljne količine folata i folne kiseline, saznanja o prehrambenim navikama žena generativne dobi vrlo su značajna. Sukladno tome, ispitan je unos folata i folne kiseline prehranom u žena starosti 20–30 godina. Također je evidentirana učestalost konzumiranja namirnica bogatih tim vitaminom kao i učestalost konzumiranja suplemenata folne kiseline. Prikazane su vrijednosti folata u serumu ovisno o njihovim prehrambenim navikama. Dobiveni rezultati ukazuju da unatoč neadekvatnom unosu folata i folne kiseline prehranom nije zabilježen klinički deficit što je rezultat učestalog konzumiranja suplemenata u prehrani.